PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Benjamin E. Reubinoff, et al.	**Examiner:**	J. T. Woitach
Serial No.:	09/436,164	**Art Unit:**	1632
Filed:	November 9, 1999	**Docket:**	13164
For:	EMBRYONIC STEM CELLS		

Commissioner for Patents
Alexandria, VA 22313-1450

DECLARATION OF MARTIN F. PERA UNDER 37 C.F.R. §1.132

Sir:

I, Martin F. Pera, hereby declare as follows:

1. I am one of the co-inventors named in the above-identified application ("the '164 application).

2. I hold a Bachelor of Arts (B.A.) Degree in English Literature and a Doctorate Degree in Pharmacology.

3. I was employed as a research scientist at the Institute of Cancer Research, London, UK, the Imperial Cancer Research Fund, London, UK, Oxford University, Oxford UK. I am currently employed by Monash University.

4. I have worked in the field of human pluripotent stem cell research since 1984.

5. I have authored over fifty publications in the field of human pluripotent stem cell research.

6. A true and correct copy of my curriculum vitae is attached hereto as Exhibit A.

7. I have read the Final Action dated December 18, 2002, and the Advisory Action dated May 1, 2003, issued in the '164 application. I have been asked to review and comment on issues raised by the Examiner in the Final Action and the Advisory Action.

8. The Examiner is of the opinion that the presently claimed methods of inducing, *in vitro*, somatic differentiation of undifferentiated, pluripotent human embryonic stem cells, are disclosed by Thomson *et al.* Science 282:1145-1147 (1998) and by U.S. Patent No. 6,200,806 to Thomson.

9. I have read and I am familiar with Thomson *et al.* Science 282:1145-1147 (1998) and U.S. Patent No. 6,200,806 to Thomson. As the Examiner has not made any distinctions between the two Thomson references, I will address the two Thomson references together (hereinafter "Thomson *et al.*").

10. Specifically, the Examiner is of the opinion that Thomson *et al.* teach the parameters that affect differentiation of ES cells – namely, the feeder layer, the cell density, and the presence or absence of various growth factors such as LIF. The Examiner contends that Thomson *et al.* teach the importance of feeder cells in differentiating ES cells. In particular, the Examiner points out that Thomson *et al.* teach that feeder cells are required for maintaining the undifferentiated state of primate and human ES cells, and that under appropriate conditions ES cells can be induced to differentiate on a fibroblast feeder layer. The Examiner also contends that it is known that if the ES cells are allowed to grow at high density, differentiation of the ES cells will occur. The Examiner concludes that the claimed methods of the '164 application do not include any steps or parameters which are different from those taught by Thomson *et al.* (See Final Action, pages 3-7; and Advisory Action, page 2). The Examiner also states in the Final Action, page 6, the last two lines, that neither the claims nor the specification of the '164 application provides any teaching regarding factors that induce cells to differentiate.

11. The '164 application is directed to an *in vitro* method of inducing somatic differentiation of undifferentiated, pluripotent human embryonic stem cells by growing the stem cells under culture conditions that preferentially induce somatic differentiation. As described in the '164 application, at page 20, lines 23 to 25, somatic differentiation *in vitro* of the ES cell

lines is a function of (i) the period of cultivation following subculture, (ii) the density of the culture and (iii) the fibroblast feeder cell layer. In the Thomson references, there is no mention that the fibroblast feeder layer is responsible for anything more that maintaining the ES cells in an undifferentiated pluripotent state. The Thomson *et al* citations do not emphasize the importance of the fibroblast feeder layer to influence the direction of differentiation after the cells are maintained in an undifferentiated state and even in a state of renewal, they remain undifferentiated and, least of all to have any influence on the advancement of the cells specifically toward a somatic lineage. The citations teach that in order to maintain a pluripotent state, the ES cell should be cultured on a (preferably) primary fibroblast feeder cell layer.

12. It is my considered scientific opinion that a principal feature of the '164 application resides in the unique recognition of the role of cell culture factors present in both the culture media and the fibroblast feeder cells for providing conditions which contribute to determining the balance between stem cell renewal in an undifferentiated state, extraembryonic differentiation, and somatic differentiation. These conditions do not kill the stem cells and do not permit continued stem cell renewal. They also do not subject the ES cells to stress which often results in unidirectional differentiation into extra-embryonic cells. The appropriate fibroblast feeder improves the differentiation event toward a somatic lineage. For example, as stated at page 20, line 19 and page 21, line 22 of the '164 application, the method of preparation and handling of the mouse embryo fibroblasts, the mouse strain from which the fibroblasts are derived, and the quality of the particular batch, may favor either one of stem cell renewal, extra-embryonic differentiation or somatic differentiation. Once a fibroblast feeder cell line that favors somatic differentiation is identified, such cell line can be stored and resurrected for subsequent use in inducing somatic differentiation, as opposed to differentiation into extra-embryonic lineages. By contrast, Thomson (US 6,200,806 column 16 line 20) teaches that "it has also been demonstrated that the particular source of fibroblasts for co-culture is not critical". In fact, on page 1146 of the Thomson *et al* in Science, he states that "Elucidating the mechanisms that control differentiation will facilitate the efficient, directed differentiation of ES cells to specific cell types."

13. It is my considered scientific opinion that the teachings of Thomson *et al.* are deficient with respect to a method of inducing somatic differentiation of undifferentiated, pluripotent human embryonic stem cells. First, although Thomson *et al.* disclose culturing

human ES cells and observe some differentiation of the cultured cells, such differentiation is uncontrolled, spontaneous differentiation with no insight as to the conditions that can influence the final outcome. These conditions were unknown to Thomson *et al* (column 16, lines 45 to 56).. Moreover, the evidence provided is completely consistent with differentiation into extra-embryonic lineages only. Thus production of alphafetoprotein or human chorionic gonadotrophin, cited as evidence of differentiation *in vitro*, likely represents the production of extra-embryonic cell types (trophoblast and extra-embryonic endoderm) rather than somatic cells. In Thomson *et al.*, there was no evidence for induction or purposeful direction of the embryonic stem cells toward somatic differentiation even after maintaining them in an undifferentiated state. Whilst in this undifferentiated state, the cells can be primed to progress toward somatic differentiation as opposed to extraembryonic differentiation. Furthermore, Thomson *et al.* do not evidence any recognition that subsets of certain types of fibroblast feeder cells support controlled differentiation of the embryonic stem cells into somatic lineages, rather than into extra embryonic lineages after maintaining cultures in an undifferentiated state.

14. In the Advisory Action, the Examiner states that while the handling or the source of the fibroblast cell line may affect its suitability as a feeder layer, there is no objective evidence of record indicating that the fibroblast layer itself uniquely impacts the differentiation of ES cells. The Examiner points out that the two fibroblast cell lines, B-83 and B-72, which were mentioned in the response to the Final Action to illustrate the suitability of fibroblast cells as feeder cells, are not identified in the specification.

15. Fibroblast cell lines B-83 and B-72 are examples of some of the cell lines that were tested for their suitability for supporting HES cell growth, undifferentiated stem cell growth and direct differentiation towards somatic lineages.

16. Several fibroblast samples were tested for their suitability. They were first derived from fresh fibroblast sources such as from fetus or embryonic or fetal tissue, as well as from sources such as human foreskin which are useful sources for fibroblasts. The sources of the fibroblasts were generally treated with minimal trypsin to provide a cell suspension. Since it was noted that trypsin may effect the fibroblasts for their ability to support HES cell growth, trypsin usage was kept to a minimum. Cells were suspended in a media of DMEM (GIBCO, without sodium pyruvate, with glucose 4500mg/L) supplemented with 20% FBS (Hyclone, Utah) β-mercaptoethanol - 0.1mM (GIBCO), Non Essential Amino Acids - NEAA 1% (GIBCO),

glutamine 2mM.(GIBCO), penicillin 50u/ml, and streptomycin 50µg/ml (GIBCO) and plated such that the final fibroblast feeder layers are at a density of approximately 25,000 to 70,000 cells per cm^2. This same media was used to culture and support ES cells on the fibroblast feeder layers.

17. The fibroblast feeders were prepared using standard protocols stated on page 15 of the '164 application. Details of the steps are outlined in Exhibit B.

18. B-72 was derived on 30 December 1998 from 12 embryos from a cross of 129Sv x 129Sv strain at day 12.5 of gestation. This cell line was derived according to the protocol outlined in Exhibit B. There was nothing unusual about the treatment of the cells and the usual three (3) incubations of 10min with trypsin was employed. B72 turned out to be a very poor feeder cell line.

19. B-83 was derived on 29 October 1999 from a cross of 129Sv x 129Sv at approximately day 13.5 of gestation which was the usual protocol unless indicated otherwise. The protocol was as described in Exhibit B except that there was only a five (5) minute incubation in item 9 and it was not repeated 3 times for 10min as with B-72. B83 served as an excellent feeder layer that could support both the propagation of undifferentiated cells and promote somatic differentiation upon prolonged culture

20. Once cells are established they were used immediately for supporting HES cell growth or they were cryopreserved. B-83 has been cryopreserved and is still in use today.

21. Fibroblasts were cryopreserved as described in '164 on page 30 under "Human stem cell cryopreservation". They may be thawed at a later date and cultured in the same manner as the fresh fibroblast feeder cells as described above.

22. Several samples were generated in the manner described above in paragraph 21 and could be tested for their suitability for supporting HES cell growth, undifferentiated stem cell growth and directed differentiation towards somatic lineages.

23. Fresh or frozen fibroblasts were plated on gelatine treated dishes at a density of approximately 25,000 and 70,000 cells per cm^2. The fibroblasts were plated up to 48 hours before culture of the stem cells in conditions as described in paragraph 16 above.

24. ES cells were propagated on the fibroblast feeder layers as described in '164 on page 29 under "Human stem cell propagation".

25. Cells were tested for their suitability for supporting HES cell growth, undifferentiated stem cell growth and directed differentiation toward somatic lineages. Importantly, these cells were "good" if they did not induce spontaneous differentiation especially toward an extraembryonic lineage. If extra-embryonic growth of the ES cells was evidenced either visually or by identification by specific markers, the fibroblast feeder cells were considered to be "bad" and thrown out. Such was the fate of the B-72 cells. The aim has always been to keep the cells in an undifferentiated state and preferably support somatic differentiation.

26. Fibroblast feeders B-83 and B-72 as well as other fibroblast feeders were tested for their suitability for supporting HES cell growth, undifferentiated stem cell growth and directed differentiation toward somatic lineages.

27. I enclose herewith Exhibit C which provides evidence as a print out from laboratory notebooks which show results from 1998 of the testing of the fibroblast cells including cell lines B-83 and B-72. The cells were assessed for not only their suitability for supporting HES cell growth, undifferentiated stem cell growth and directed differentiation toward somatic lineages, but also whether the frozen samples were just as effective as fresh fibroblasts and whether the freezing did affect performance.

28. In the table below I summarize points of note from Exhibit C.

Box Identification	**Sample**	**Comment**
Box 65a, HES BACK UP	B-49	This sample was frozen and tested on 24 August 1998
Box 2, Cane 1	B-74	These cells were "good as fresh" and hence determined to be particularly suitable as fresh fibroblasts that can be further passaged and used.
Box No8, Cane 5	B-75	Determined to be "good" as fresh fibroblasts.
	B-79	Determined to be "good" as fresh fibroblasts
	B-73	Determined to be "good" as fresh fibroblasts but if frozen/thawed they were only moderate.
Box 1, Cane 5	**B-72**	**Determined as "bad" and not suitable and discarded**
Box 7, Cane 1	B-75	Determined to be "good" as fresh fibroblasts

Box 7, Cane 6	B-71	Determined to be "good" as fresh fibroblasts
	B-60	Determined to be "good" as fresh fibroblasts
	B-61	Not suitable
	B-65	Not suitable-discarded
	B-73	Determined to be "good" as fresh fibroblasts but "poor" as thawed
	B-69	Determined to be "good" as fresh fibroblasts but prolonged passaging deteriorated the fibroblasts
Box 6, Cane 1	B-81	Not suitable and discarded
Box 1 (1/1), Cane 1	**B-83**	**This seen as "good" as fresh or thawed fibroblasts. These fibroblasts are still in use.**

29. Fibroblast feeders were again tested in May 1999. I enclose herewith Exhibit D which shows hand written results of testing in May 1999. B-83 is still seen to be "good" and we are still using this fibroblast feeder sample today.

30. By contrast, Exhibit E shows a colony of HES cells grown on human foreskin feeder cells designated Hs27. The Hs27 cells were produced using a similar procedure to that outlined in Exhibit B. This colony has differentiated into flattened epitheleioid cells which form some cystic structures, a morphology typical of extraembryonic endoderm differentiation. This feeder layer would not be suitable as a differentiation inducing fibroblast feeder layer to induce a differentiated somatic lineage or multiple differentiated somatic lineages.

31. The results in Exhibits B, C, D and E clearly illustrate that the fibroblast layer uniquely impacts the differentiation of HES cells, and that fibroblast feeder cells differ in their potential to support undifferentiated stem cells and induce controlled differentiation of the embryonic stem cells into somatic lineages. Several lines need to be tested to be determined as suitable and once established can be frozen and thawed and used providing the criteria of supporting HES cell growth, undifferentiated stem cell growth and directed differentiation towards somatic lineages can be satisfied. Thus, identification and selection of suitable fibroblast feeder cells permit effective culturing conditions which maintain cells in an

undifferentiated state and can be induced to differentiate toward a somatic lineage, as opposed to differentiation into extraembryonic lineages.

32. In sum, it is my considered scientific opinion that the recognition of the effect of cell culture conditions on the extent of, extra-embryonic differentiation, or stem cell renewal, including such conditions as the fibroblast feeder layer and the application of inhibitors of extra-embryonic differentiation, such as bone morphogenetic protein-2 or antagonists of such factors , are uniquely provided by the '164 application, and are not taught by Thomson *et al.* or any prior art extant.

33. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that those statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

By: ______________________ Dated: 18/12/2003

PATENTS

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Serial No.:	09/436,164	**Art Unit:**	1632
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DECLARATION OF MARTIN F. PERA UNDER 37 C.F.R. §1.132

This is the exhibit marked as "Exhibit A" referred to in the Declaration of MARTIN F. PERA.

By: ______________ Dated: 18/12/2003

CURRICULUM VITAE

MARTIN F PERA

DATE OF BIRTH: 16 July 1951

PLACE OF BIRTH: Chicago, Illinois, U.S.A.

NATIONALITY: U.S. Citizen, permanent resident status in Australia pending citizenship

STATUS: Married to Dr. Gillian Duchesne, Professor and Director of Radiation Oncology, Peter McCallum Institute. Daughter Virginia by previous marriage

WORK ADDRESS: Monash Institute of Reproduction and Development
27-31 Wright Street
Clayton, Victoria 3168 Australia
Phone +61 (0)3 9594 7318
Fax +61 (0)3 9594 7311
email martin.pera@med.monash.edu.au

HOME ADDRESS: 53 Highbury Grove
Prahran East
Victoria 3181 Australia
Phone and Fax +61 (0)3 9529 1560

QUALIFICATIONS: **B.A.**, English Language and Literature (1972), College of William and Mary, Williamsburg, VA. U.S.A.
Ph.D., Pharmacology (1979), George Washington University, Washington, D.C. U.S.A.
M.A. (1990) Oxford University, Oxford, U.K.

EMPLOYMENT: **1979-1982** National Institutes of Health Postdoctoral Fellowship (National Research Service Award); Institute of Cancer Research, London, U.K.
1982-1984 Research Fellow, Imperial Cancer Research Fund, London, U.K.
1984-1988 Cell Biologist, Institute of Cancer Research, Sutton, Surrey, U.K.
1989- 1996 Group Leader, Cancer Research Campaign, Department of Zoology, Oxford University, Oxford, U.K.
Sept 1996- Sept 1997 Senior Research Fellow, Monash Institute of Reproduction and Development, Monash Medical Center
Sept 1997-2001 Deputy Head, Centre for Early Human Development

June 2000 – Associate Professor, Centre for Early Human Development
July 2001-Dec 2002 Co-Director, Centre for Early Human Development
Jan 2003-present Director, Centre for Early Human Development
1996-2000 - Consultant, Stem Cell Sciences Pty. Ltd
2000-2002 - Founding Scientist and member of the Scientific Advisory Board ESI Pte. Ltd.
Founding scientist, Copy Rat Pty. Ltd.
Director, Biotrophix Pty. Ltd.
2003 – Member Scientific Management Advisory Committee (National Stem Cell Centre, NSCC)

PROFESSIONAL SOCIETIES:

British Society for Cell Biology
British Society for Developmental Biology
Australia/New Zealand Society for Cell and Developmental Biology
International Society for Stem Cell Research

RESEARCH INTERESTS:

Human embryonic stem cells and human development; control of growth and differentiation of human pluripotent stem cells; human germ cell tumours of the testis

AWARDS AND HONOURS

National Institutes of Health (U.S.) National Research Service Award, 1979
M.A., Oxford University, 1990
Senior Scientist, Monash Institute of Reproduction and Development, 1997

TEACHING AND ADMINISTRATIVE RESPONSIBILITIES (From 1996)

Teaching

Lecture to Masters of Reproductive Science program, Monash University.

Supervision of Minor Projects in Physiology, Monash University.

Deputy Director and Course Module Co-ordinator, Masters of Clinical Embryology, Monash University. Development of this Course Module. Module MCE 101, Introduction to Mammalian Development

Lecturing in MCE101, Introduction to Mammalian Development

Supervision of Master of Reproductive Science projects (Mr. Tim Xiang, January 1998-March 1999 Mr. Gary Peh 2002 – 2003).

Supervision of Honours project (Ms. Emma Langton-Bunker, from March 1998-November 1998, Mr. Ben Rollo, from February 2000-Feb 2001, Mr. Adam Filipczyk, from February 2000-Feb 2001) Mr Hayden Waterham (From Feb 2001-Feb 2002) Mr. Lincon Stamp July 2002 – July 2003. Mr. Raymond Wong from January 2003 - November 2003

Supervision of Ph.D. students (Dr. Ben Reubinoff, from February 1998-2001). Ms Jessica Andrade from June 1998-June2003. Ms. Carmel Obrien from August 1998 Mr Adam Filipczyk from April 2001-April 2005. Ms. Kathy Davidson from January 2003 – December 2006. Ms. Elizabeth Stadler from July 2002 – July 2010. Ms Anna Mossman March 2003 – March 2007

Appointments and administrative duties

Director, Centre for Early Human Development
Co-Director, Centre for Early Human Development
Chair of Senior Scientist Forum, Monash Institute of Reproduction and Development.
Student Advisor, Centre for Early Human Development

Committees

1996-2003
Annual Report Committee, MIRD
Cloning Symposium Committee, MIRD
Building Committee, MIRD
Postgraduate Student committee, MIRD
Chair, Equipment Committee, MIRD
Chair, Senior Scientists Forum, MIRD

2002-2003
Research Degrees Committee, Monash University (from 2002)

Summary of Research, Management Responsibilities and Funding

1974-1979: Ph.D. Student, Department f Pharmacology, George Washington University, Washington, D.C.

Studied the pharmacology and toxicology of the antitumour agent cisplatin; demonstrated protection from renal toxicity and an improved therapeutic index when the drug was used in combination with diuretics in rodent model systems.

1979-1982: N.I.H. National Research Service Award, Institute of Cancer Research, London, U.K.

Carried out molecular pharmacological studies of cisplatin which provided strong evidence for the role of DNA binding and repair in the response of normal and tumour cells to the drug.

1982-1984: Postdoctoral Fellow, Imperial Cancer Research Fund, London, U.K.

Developed a new culture system for normal mouse keratinocytes and keratinocytes from preneoplastic and malignant stages of skin carcinogenesis in the mouse. Proved that immortality and reduced growth factor dependence preceded malignant transformation in skin tumour development. Showed that resistance to the induction of terminal differentiation by tumour promoting phorbol esters was a characteristic of premalignant keratinocytes derived from papillomas.

Management responsibilities: supervision of one technician

1984-1989: Cell Biologist, Institute of Cancer Research, Sutton, U.K.

Established and characterised a panel of cell lines from human germ cell tumours which constitute a unique resource for the study of growth and differentiation in early human embryogenesis and in testicular tumours. Produced new monoclonal antibodies for the analysis of cell differentiation lineage in these neoplasms. Obtained evidence for the critical role of the serum adhesion protein vitronectin in the growth of human germ cell tumours. Demonstrated that germ cell tumours were inherently sensitive to DNA damage induced by cisplatin. Collaborated on the development and characterisation of a panel of human lung carcinoma cell lines.

Management responsibilities and funding: Head of a team within the Section of Radiotherapy in the Institute of Cancer Research. Supervision of one technician, Ph.D student, M.D. student, one postdoctoral fellow. Four year program reviewed and approved by UK Cancer Research Campaign; also two two year grants from the Bob Chamion Cancer Trust.

1989-1996: Group Leader, Department of Zoology, Oxford University, Oxford, U.K.

Played a major role in international collaborative studies on the phenotypes and cell lineages of human germ cell tumours, and on the basis of their sensitivity to cytotoxic drugs. Discovered a new keratan sulphate proteoglycan expressed in human embryonal carcinoma, other tumours, and foetal tissues; reported on the

purification, biochemical characterisation, and tissue distribution of this new pericellular matrix molecule; showed its potential use as a serum tumour marker. Carried out cell and molecular studies of gene expression during retinoic acid-induced differentiation of multipotent human embryonal carcinoma stem cells into endoderm. Characterised a novel polypeptide factor which promotes growth and inhibits differentiation of multipotent embryonal carcinoma stem cells. Identified CD30 and CD30 ligand as potential autocrine regulators of human embryonal carcinoma stem cells. Collaborated with several groups to examine the expression of activin and related molecules in human germ cell tumours, work which led to the identification of human GDF-3 as a stem cell marker and possible growth regulator.

Management responsibilities and funding: Head of a team within a UK Cancer Research Campaign Programme Grant. Supervision of postdoctoral fellow, 2 technicians, 2 Ph.D. students and 5 undergraduate projects. Coinvestigator on 5 year programme grant "Human teratoma and growth regulation" to Professor Chris Graham from UK Cancer Research Campaign. (1990-1996, UK sterling350,000 per annum) Co-investigator on NATO grant to support international conference on germ cell tumours in our department (1992, US$50,000).

Sept 1996-present: Senior Research Fellow, Monash Institute of Reproduction and Development, Monash University, Clayton, Victoria

Developed novel assay for human multipotent stem cell factor which will allow expression cloning of this molecule. Completed studies on induction of differentiation of human pluripotent stem cells by BMP-2. Completed collaborative study on novel forms of Leukemia Inhibitory Factor produced by human germ cell tumours. Completed collaborative study on cytogenetic changes in cell lines from human germ cell tumours. Carried out further studies elucidating role of CD30 and its ligand in human germ cell tumours which demonstrated production of truncated form of the receptor in these cells and established a role of CD30 in stem cell survival. Directed and participated in project which resulted in establishment and characterisation of human embryonic stem cell lines from blastocysts and the derivation of pure cultures of neuronal progenitors therefrom. Carried out research on spontaneous differentiation of human embryonic stem cells identifying BMP-2 as a molecular mediator and noggin as an inducer of stem cell differentiation.

Present activities: Development of new culture systems for human embryonic stem cells and study of growth factors involved in stem cell maintenance, cell cycle traverse and survival. Characterisation and control of early differentiation of human embryonic stem cells. Isolation, propagation and characterization of neural progenitor cells and endodermal progenitor cells from human embryonic stem cells. Isolation of early precursors of nephrogenic mesenchyme from early human ES cells. Development of high throughput methodology for screening of factors active on human pluripotent stem cells.

Management responsibilities and funding: Establish and co-ordinate a new section in the Centre for Early Human Development of the IRD, "Human Embryonic Cell Biology". Deputy Head of the Centre for Early Human Development and Deputy Director of the new Master's in Clinical Embryology Program. Co-Director, Centre for Early Human Development, Director from January 2003.Funding from Stem Cell Sciences Pty., Australian Research Council, Monash IVF. Equipment grants from Rebecca L. Cooper Memorial Foundation and the Wellcome Trust. Project grant

beginning 1999 from National Health and Medical Research Council. Co-investigator, Monash University Special Research Fund grant: Centre for Animal Clonal Genomics for Biomedicine. Chief Investigator, Monash University Special Research Fund grant: Human embryonic stem cells in biology and medicine. Founding scientist, ES Cell International Pte & Copy Rat Pty. Chief Investigator, ESI Research Contract from 2000-2003. Chief Investigator, NHMRC/JDRF Program Grant from 2000-2005. Chief Investigator, NHMRC project grant from 2002. NIH Kidney consortium. NIH project grant. Program Leader on successful Biotechnology Centre of Excellence bid to establish the National Stem Cell Centre. Member, Scientific Management Advisory Council of the National Stem Cell Centre. Provide advice to national, state and foreign government and regulatory agencies on therapeutic cloning and embryonic stem cells. Member, Victorian Government Working Party on Stem Cell Research. Provide public commentary on scientific and ethical aspects of research on human embryonic stem cells and therapeutic cloning.

Refereeing of grants and manuscripts

Grant reviews for NHMRC, ARC, UK BBSRC, UK MRC, Wellcome Trust, Yorkshire Cancer Research Campaign, Israel Science Foundation, other international bodies
Manuscript referee for PNAS, International Journal of Cancer, Experimental Cell Research, Science, International Journal of Radiation Biology,Reproduction Fertility and Development (Editorial Board), Nature, Stem Cells, Nature Biotechnology, Molecular Reproduction and Development, Developmental Biology, Journal of Anatomy, the Journal of Cell Science, Current Biology, Mechanisms of Development, and others

Patents

Pera MF. (1998). Growth Factor. Australian Provisional PP2609 with Stem Cell Sciences.

Reubinoff, B., Trounson, A.O., Pera, M.F. Chui, F.Y. and Bongso, A. (1998). Embryonic Stem Cells. Australian Provisional PP7009.

Reubinoff B Pera, M Trounson AO Chui F and Bongso A 2000. Embryonic stem cells and neural progenitors derived therefrom. Australian Provisional PQ6211.

Pera MF. Improved methods of culturing embryonic stem cells. Australian Provisional. 2000.

Reubinoff, B. Ben-Hur T and Pera MF. Embryonic stem cells and neural progenitors derived therefrom. Australian Provisional 2000.

Pera MF. Characterisation and Isolation of Subsets of human embryonic stem cells and progenitor cells derived therefrom. Australian Provisional 2001.

Andrade JM Herszfeld D and Pera MF. Regulation of human pluripotential cells by bone morphogenetic protein 2 and antagonists. US patent application 09/670198

International Applications

1. ***Embryonic Stem Cells***

PCT	AU99/00990	**Filing Date:**	9/ 11/ 99
US Application No	09/436164	**Filing Date:**	9 /11/ 99
Priority Date:	9 November 1998 (PP 7009) 15 September 1999 (PQ 2852)		
Inventors:	**B. Reubinoff, M. Pera, A. Trounson, A. Bongso, C. F. Yee**		
National Phase Entry	Singapore 6 April 2001 – patent granted Israel 23 April 2001 Canada 4 May 2001		
• **Activity:**	USA claims restrictions filed with the US office – responses filed to examiner objections and we await a response Divisional application progressing – no recent activity		

2. ***Embry nic Stem Cells and Neural Progenitor Cells deriv d there from***

PCT	AU01/00278	**Filing Date**:	14 /03 / 01
US Application:	US09/808362	**Filing Date**:	14 /03/ 01
Priority Date	March 14 2000 (PQ 6211) 46 November 2000 (PR1279) 6 February 2001 (PR 2920)		
CIP Filing Date:	4 October 2001		
Inventors:	**M. Pera, B. Reubinoff, T. Ben-Hur**		

- **Activity:** Claim amendments (reduction) achieved – await further correspondence from the examiner.

*3. **Embryonic Stem Cells and Neural Progenitor Cells derived therefrom – Continuation***

Filling Date:	4 October 2001		
US Application:	09/970,543	**Filling Date:**	**04/10/01**
Priority Date:	March 14, 2000 (PQ 6211) 6 November 2000 (PR1279) 6 February 2001 (PR2920)		
Inventors:	**M Pera, B. Reubinoff, T.Ben-Hu**		

*4. **Improved Methods of Culturing Embryonic Stem Cells (Noggin)***

PCT	AU01/00735	**Filing Date**:	**20/06 /01**
US Application	US09/835679	**Filing Date**:	**20 06 /01**
Priority Date:	20 June 2000 (PQ 8242) 8 November 2000 (PR1327) No Activity this period		
Inventors:	**M. Pera**		

*5. **Characterisation and isolation of subsets of human embryonic stem cells and progenitor cells derived therefrom***

PCT	PCT/AU02/01534	**Filling Date:**	**11 /11/02**
US Application:	TBA		
Priority Date:	15 March 2002 (PS1129) 9 Nov 2001 (PR8781)		
Inventors:	**M Pera, A Laslett, S Hawes**		

Provisional Patent Applications

PR8781 – Filed 9 November 2001 – **Characterisation and isolation of subsets of human embryonic stem cells and progenitor cells derived therefrom**

(Also to include **PS1129** – Filed 15th March 2002)
Inventors: M. Pera, A. Laslett, S. Hawes

PS2819 – filed 6th June 2002 - **Differentiation of HES Cells – (Pancreatic Islet cells)**

- Discloses methodology for the production of Pancreatic Islet cells from ES Cells.
- Inventors: T. Gion, M. Pera
- This is new data

PS2860 - filed June 7th 2002 – **Methods f regulating differentiation of Stem Cells, (S1P)**

- A small molecule (phospholipid) that can limit the differentiation of ES cells – a "differentiation factor"
- Inventors: - A. Pebay, M.Pera

PS2861 – filed June 7th 2002 - **Screening method** – (**S1P** – Pebay, Pera)

- The screening method used in the above application – first method described that can screen for Edg Receptor agonists on HES cells.
- Inventors: - A. Pebay, M. Pera

2003901313 – filed 21 March 2003 – Undifferentiated Cells (PDGF – Pebay, Pera)

- New PDGF data
- Investors: - A Pebay, M Pera

TBA
- filed 26 May 2003 – Undifferentiated Cells (2) (PDGF – Pebay, Pera)
- New PDGF data
- Investors: - A Pebay, M Pera

Grants obtained at Monash University (* Chief Investigator)

*Rebecca L. Cooper Medical Research Foundation. $10, 695. In vitro model for investigating the effects of genetic abnormalities on early human development.

Monash IVF. Increasing IVF success rates by optimisation of culture conditions to produce viable blastocysts for transfer and cryopreservation. $2500.

*Stem Cell Sciences Pty. Ltd. Research Agreement. $75,000 1997

*Stem Cell Sciences Pty. Ltd. Research Agreement. $75,000 1998

Wellcome Foundation Equipment Grant. $200,000. 1998.

*Monash IVF. Cell biology of human peri-implantation development. $20,000. 1998

*ARC, APAI award, $20,502. 1998.

*NHMRC Project Grant: Combinatorial Regulation of Human Multipotent Stem Cells by Membrane Bound and Soluble Factors. $110,000 in 1999 (for three years).

Monash University Special Research Fund. Centre for Animal Clonal Genomics for Biomedicine ($160,000; one year 1999).

*Monash University Special Research Fund. Human embryonic stem cells in biology and medicine ($160,000; one year 1999).

*ESI. Growth and Differentiation of human embryonic stem cells (2000-2001, $525,000)

*ESI. Growth and Differentiation of human embryonic stem cells (2001-2003, $900,000 per annum)

NHMRC/JDF Program Grant. Creating Islet cells to cure Type I diabetes. (2001-2006). $825,000 per annum.

*NHMRC Project Grant Control of growth and differentiation of normal and malignant pluripotent human stem cells (2002-2005, $160,000 per annum)

*Monash University SMURF 2, "Development of square wave technologies for human embryonic stem cells and other cell types" Equipment grant, $30,000. 2001.

*National Institute of Health (NIH) Research Grant – "Towards Renal Regeneration" (2002-2005, US$99,000)

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* Pera, MF Johnson, J Reubinoff B Houssami S Herszfeld D Andrade J and Trounson A. Human Pluripotent Stem Cells. ICDCB, Gold Coast, Sept 2000.

The role of CD30 in the maintenance of malignant human pluripotent stem cells. Daniella Herszfeld[1], Emma Langton -Bunker[1], Benjamin E. Reubinoff[1,2], Souheir Houssami[1], Leendert H.J. Looijenga[3], and Martin F. Pera[1]. ICDCB Gold Coast, Sept 2000.

BMP-2 regulation of the differentiation of human pluripotent stem cells. Jessica Andrade[1], Jacqui Johnson [1], Benjamin E. Reubinoff[1,2], Souheir Houssami[1], Alan Trounson[1], and Martin F. Pera[1]. ICDCB Gold Coast, Sept 2000

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*Pera MF. Human pluripotent stem cells. Australian Flow Cytometry Group, Melbourne November 2001.

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*Pera MF Human embryonic stem cells. March 2002 Taiwan Society of Obstetrics and Gynecology, Tapei.

*Pera MF Human embryonic stem cells: work in progress. June 2002 NIGMS Symposium on the Basic Biology of Pluripotent Stem Cells. Bethesda Md.

*June 2002 Australian Society of Nephrology

*Pera MF August 2002 Jackson Laboratory course Current Protocols in Stem Cell Biology Faculty. Maine August 2002

* Pera MF MDIBL stem cell conference Stem Cells by Land and Sea. Maine August 2002.

*Pera MF Embryonic stem cells. Sept 2002 Haematology Society of Aust & NZ, Annual Scientific Meeting – Adelaide SA

2002 COMBI02002, Sydney NSW
A Filipczyk, A. L. Laslett, S. H. Houssami & M.F. Pera.
Effects of Insulin Function in supporting the growth of Human Embryonic Stem Cells

*Pera MF Human embryonic stem cells:characterization growth and differentiation. October 2002 33rd Paterson Institute Conference Stem Cells Manchester UK

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*Pera MF Embryonic stem cells. Dec 2002 American Society of Hematology – Philadelphia 2002.

*Jan 2003 Forum on Spinal Cord Injury and Conditions, Sydney NSW

Mar 2003 The Croucher Foundation Advanced Study Institute (ASI), Hong Kong
Advances and Challenges of Stem Cell Research

Martin F. Pera, Jessica Andrade, Susan Hawes, Souh ir Houssami, Andrew Laslett, Alice Pebay, and Tomonbu Gion

Human embryonic stem cells: pluripotent cells and their progeny Mar 2003
Keystone Symposia from Stem Cells to Therapy – Colorado

April 2003 Stem Cell Workshop - Pittsburgh

May 03 – Reality Check - A Summit of Young Adults with diabetes – Melbourne
Stem Cell Research Update

May 22 – St George Hospital – Sydney NSW

May 26– CSIRO Horizons in Livestock Sciences – Seaworld Nara Resort – Qld
The impact of the new biology Embryonic stem cells: a future in agricultural biotechnology?

May 30 – Australian Society for Medical Research - UK

June 04 – 2nd Stem Cell Workshop, The Prince of Wales Hospital, Sydney NSW
Potential Clinical Applications of Stem Cells for Neurological disorders

June 04 – Australian Society of Medical Research Seminar – Sydney NSW
Therapeutic Cloning

June 09 - Culture techniques for human embryonic stem cells.– NIH Stem Cell Symposium and Workshop Washington DC

July 04 – CSIRO Conference – Melbourne Convention Centre

July 13 – Diabetes One – JDRF Research Symposium and Expo – Perth
Latest Developments in Stem Cell Research incl. New techniques to turn mouse and human embryonic stem cells into insulin producing cells

July 17 – Towards Renal Regeneration Symposium – University of Queensland
Pushing Human Embryonic Stem Cells towards Mesoderm

August 3 to 8 – Current Protocols in Stem Cell Biology – The Jackson Laboratory – Bangor – USA

September 1 – Peter MacCallum Clinical Grand Rounds 2003 – Peter MacCallum Cancer Institute – East Melbourne
Human Embryonic Stem Cells: Progress & Future Prospects

Sept 5 to 7 – Stem Cells: From Genetics to Cell Therapy - Lund Stem Cell Center – Sweden

OIPE JAN 2 0 2004 JC10 PATENT & TRADEMARK OFFICE

PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Benjamin E. Reubinoff, et al.	**Examiner:**	J. T. Woitach
Serial No.:	09/436,164	**Art Unit:**	1632
Filed:	November 9, 1999	**Docket:**	13164
For:	EMBRYONIC STEM CELLS		

Commissioner for Patents
Alexandria, VA 22313-1450

DECLARATION OF MARTIN F. PERA UNDER 37 C.F.R. §1.132

This is the exhibit marked as "Exhibit B" referred to in the Declaration of MARTIN F. PERA.

By: ______________ Dated: 18/12/2003

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EXHIBIT B

Protocol for preparing fibroblast cells

1. Sacrifice pregnant mice following superovulation. F1 c57/black6 x CBA was sometimes good. 129/Sv x c57/black6, 129/Sv x 129/Sv, CBA-CaH x CBA-CaH were all used. 129/Sv x 129/Sv from day 13.5 form good well developed embryos. Cobel stone appearance of cells which are rapidly dividing are probably the best.
2. Remove the two uterine horns under sterile conditions into PBS.
3. Remove 12-14 embryos from the uterus and from the amniotic sac.
4. Allow embryos to bleed and then wash as much blood as possible by transferring the embryos into a new 10cm petri dish with fresh PBS.
5. Transfer embryos again into a new 10cm petri dish with PBS and remove heart, lungs, liver, internal organs and upper head.
6. Wash embryos in 10cm petri dish with 10ml PBS x3.
7. Transfer embryos into 6cm petri dish with small amount (200ml) of PBS and mince with two round scalpel blades (No 20).
8. Transfer cells in Trypsin/EDTA 0.25%/0.04% into 50ml falcon tube. Add 2ml solution/embryo and up to 5 embryos/tube = 10ml.
9. Incubate with shaking 10 min in water bath at 37°C.
10. Add 40ml mouse embryonic fibroblast (MEF) medium to the tube.
11. Allow large pieces to settle down and remove supernatant. Large pieces can undergo another two rounds of trypsinization as in items 8-11.
12. Count cells and plate 30,000,000 per T-175 (usually 5 embryos / T-175)
13. Change medium after about 4 hours.
14. Change media the following morning.
15. After 8 hours, if confluent, trypsinize cells (0.05% trypsin) and split each T-175 into 6 x 10cm petri dish (about 1:2 dilution) or two T175 (P0). If the MEF are not confluent after 8 hours, wait for confluence.
16. When MEFs are confluent freeze each plate into a single ampoule or each flask into 2 ampoules (primary stock).
17. Keep one T-175 ongoing, expand 1:3 (P1) and freeze again.

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JC102
PATENT & TRADEMARK OFFICE

PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Benjamin E. Reubinoff, et al.	**Examiner:**	J. T. Woitach
Serial No.:	09/436,164	**Art Unit:**	1632
Filed:	November 9, 1999	**Docket:**	13164
For:	EMBRYONIC STEM CELLS		

Commissioner for Patents
Alexandria, VA 22313-1450

DECLARATION OF MARTIN F. PERA UNDER 37 C.F.R. §1.132

This is the exhibit marked as "Exhibit C" referred to in the Declaration of MARTIN F. PERA.

By: ________________ Dated: 18/12/2003

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BOX 65a. AC~~[illegible]~~3RCA.

HES BACKUP

	1	2	3	4	5	6	7	8	9	10
A	HES-1 5.11 P6	HES-1 5.11 P6	HES-1 5.11 P6	HES-1 8.11 P6	HES-1 8.11 P6	HES-1 8.11 P6	HES-1 11.11 P6	HES-1 11.11 P6	HES-1 11.11 P6	HES-1 13.11 P7
B	HES-1 13.11 P7	HES-1 17.11 P7	HES-1 17.11 P7	HES-1 18.11 P6	HES-1 18.11 P7	HES-1 18.11 P7	HES-1 19.11 P8	HES-1 19.11 P8	HES-1 24.11 P7	HES-1 24.11 P7
C	B-47 MEF	B-47 MEF	B-47 MEF	B-47 MEF	B-47 MEF	B-47 MEF	B-48 MEF	B-48 MEF	B-48 MEF	B-48 MEF
D	B-48 MEF	B-48 MEF	B-48 MEF	B-49 MEF	B-49 MEF	B-49 MEF	B-49 MEF	B-49 MEF	B-49 MEF	
E	B-50 MEF	B-50 MEF	B-50 MEF	B-50 MEF	B-50 MEF	B-50 MEF	B-50 MEF	B-50 MEF	B-51 HEF	
F	~~B-51 HEF~~	B-51 HEF	B-51 HEF	B52 HEF	B52 HEF	B52 HEF	B52 HEF	B52 HEF	HES-2 31.3	
G	B54 HEF	B54 HEF	B55 HEF	B55 HEF	B56 HEF	B56 HEF	B56 HEF	B56 HEF	B56 HEF	B56 HEF
H	B57 HEF	B57 HEF	B58 HEF	B58 HEF	B58 HEF	B-59 MEF	B-59 MEF	B-59 MEF	B-59 MEF	
I	B-59 MEF	B-59 MEF	HES-2 31.3							
J										

B-47	21.8	F1 P0 4x175 → 7 AMP
B 48	22.8	F1 P0 OVERCROWDED 4x175 → 7AMP
B 49	24.8.98	HEF I P9 3x T175 → 6 AMP
B 50	3.10	B-44 → THAWED TO P4 3x T-175 + 2x75 → 8XAMP
B 51	5.10	HEF I THAWED P4 → FREEZE P7 2x175 + 1x75 — 5XAMP
B 52	13.10	HEF I THAWED P4 → FREEZE P7 5 AMP
B 54	15.10	HEF I THAWED P4 → FROZEN P7 2 AMP
B 55	16.10	HEF II P7 1x175 → 2AMP
B 56		HEF II 3x175 → 6 AMP P8
B 57		HEF II 1x175 → 2 AMP P9
B 58		HEF II 1x175 + 1x75 → 3XAMP P10
B 59		MEF F1 2x175 + 2x75 → 6XAMP P2

BOX 2 CANE 1

B-74 P0 6.1 B-74 7 P0

	1	2	3	4	5	6	7	8	9	10
A	ARIF F	ARIF F	ARIF F	ARIF F	~~MEFM B-39~~	~~MEFM B-39~~	~~MEF MB-39~~	B-74 6.1	B-74 6.1	~~B-74 6.1~~
B	B-41 MEF	B-41 MEF	B-41 MEF	B-41 MEF	B-41 MEF	B-74 6.1	HES-1 13.1.9	HES-1 15.1	HES-1 15.1	HES-1 22.1
C	TRA-2 m-42 11.8.98	TRA-2 m-42 11.8.98	TRA-2 m-42 11.8.98	TRA-2 m-42 11.8.98	TRA-2 m-42 11.8.98	TRA-2 m-42 11.8.98	TRA-2 m-42 11.8.98	TRA-2 m-42 11.8.98	TRA-2 m-42 11.8.98	TRA-2 m-42 11.8.98
D	B-32 12.8	B-32 12.8	B-32 12.8	B-32 12.8	B-32 12.8	B-32 13.8	B-32 13.8	B-32 13.8	B-32 13.8	
E	B-30 OK01	B-30 OK01	B-30 OK01	B-30 OK01	B-30 OK01	B-30 OK016	B-30 OK01	B-30 OK016	B-30 OK01	~~B-30 OK01~~
F	B-30 OK01	B-30 OK01	B-30 OK01	B-30 OK01	B-30 OK01	B-30 OK016	B-30 OK01	B-30 OK016	B-30 OK01	B-30 OK01
G	B-32 9.6	B-32 9.6	B-32 4.6	B-32 10.6	B-32 12.6	B-32 25.6	B-32 20.8	B-32 20.8	B-32 20.8	B-32 20.8
H	MEFT B-38	MEFT B-38	MEFT B-38	MEFT B-38	MEFT B-38	MEFT B-38	MEFT B-38	MEFT B-38	MEFT B-38	MEFT B-38
I	MEFT B-38	MEFT B-38	MEFT B-38	MEFT B-38	MEFT B-38	~~MEFM B-39~~	~~MEF MB-39~~	~~MEFM B-39~~	~~MEF MB39~~	~~MEF MB-39~~
J	~~MEFM B-39~~	~~MEFM B-39~~	~~MEFM B-39~~	~~MEFM B-39~~	~~MEFM B-39~~	~~MEFM B-39~~	~~MEFM B-39~~	~~MEFM B-39~~	~~MEFM B-39~~	~~MEFM B-39~~

ARIF = OLD STUFF FROM ARIFF

NAME	DATE	DETAILS
B-39	16.6	T-175 x 6 → 18 AMP, P_1 F1 - MECHANICAL DERIVA[TION] USED?
B-74	6.1	2x 175 → 4 AMP, P_0 GOOD AS FRESH, 129X C57
B-41	17.6	B-34 that has been further propagated P_2
B-32		RHESUS CELLS
B-38	11.6	5x 175 → 15 AMP P_1 F1
B-39		SEE ABOVE

BOX No 8 CANE 5

	1	2	3	4	5	6	7	8	9	10
A		B-73 8.1.99	B-73 8.1.99	B-73 8.1.99	B-73 8.1.99	B-73 8.1.99	M-21 P3T3	M-21 P3T3	M-21 P3T3	M-21 P3T3
B	B-74 9.1.99	M-22 72	M-22 72	M-22 72	M-22 72	M-22 72	M-22 72	M-22 72	M-22 72	M-22 72
C		M-23 72	M-23 72	M-23 72	M-23 72	M-23 72	M-23 72	M-23 72	M-23 72	M-23 72
D	M-24 1B9	M-24 1B9	M-24 1B9	M-24 1B9	M-24 1B9	M-28 F9	M-28 F9	M-28 F9	M-28 F9	M-28 F9
E	M-25 44	M-25 44	M-25 44	M-25 44	M-25 44	M-25 44	M-25 44	M-25 44	M-25 44	M-25 44
F	B-32 26.5	B-32 27.5	B-32 27.5	B-32 28.5	B-32 28.5	B-32 29.5	B-32 29.5	B-32 29.5	B-32 1.6	B-32 3.6
G	M43 293T	M43 293T	M43 293T	M43 293T	M43 293T	B-74 9.1.99	B-74 9.1.99	B-74 9.1.99 P2	B-74 9.1.99	B-74 9.1.99
H	M41 293T	M41 293T	M41 293T	M41 293T	M41 293T	M41 293T	M41 293T	M41 293T	M41 293T	M41 293T
I	M-26 85.1	M-26 85.1	M-26 85.1	M-26 85.1	M-29 F9	M-29 F9	M29 F9	M29 F9	M29 F9	M29 F9
J	M-27 85.2	M-27 85.2	M-27 85.2	M-27 85.2	M27 85.2	B-75 23.1	B-75 23.1	B-75 23.1	B-75 23.1	B-75 23.1

NAME	DATE	DETAILS
B-75	23.1	P2, 2x175 + 1x75 → 5 AMPULES (5 DAYS IN -80) C57x129 GOOD AS FRESH
B-74	9.1	P2, 3x 175 → 6X AMP C57x129 GOOD FUNCTION AS FRESH
B-73	8.1	P2, 3x175 → 6X AMP GOOD AS FRESH MODERATE AS THAWED
B-32		Rhesus Cells

BOX 1 CANE 5

	1	2	3	4	5	6	7	8	9	10
A	70 AB3	w209 w	w169	355	2125	tera2	474	2121	2162	TN/ 1H1
B	Z42	Z218	Z227	355	87B1	Z74	Tera2	NEK 8Z	357	796
C	993	539	202	188	355	818	w116	z218	x131	x74
D	z236	z53	z854	z013	z126	z13	466	x234	z165	244
E	223	x22	x200	w230	x74	z43	w215	125	227	43
F	539	236	128	499	42	22	74	202	400	81
G	796	253	234	218			~~B-46 15.8~~	B-46 15.8	B-46 15.8	B-46 15.8
H	B40 ~~mefT~~	B40 ~~mefT~~	B40 ~~mefT~~	B40 ~~mefT~~	B40 ~~mefT~~	B40 ~~mefT~~	~~B45-α~~ 10.8	~~B45-α~~ 10.8	B45-α 10.8	B45-α 10.8
I		B44 ~~mef-C~~	B44 ~~mef-C~~	B44 ~~mef-C~~	B44 ~~mef-C~~		B45-D ~~10.8~~	B45-D ~~10.8~~	~~B45-D~~ 10.8	~~B45-D~~ 10.8
J	M44 Tera2	M44 Tera2	M44 Tera2	M44 Tera2	M44 Tera2		B-72 2.1.99	B-72 2.1.99	B-72 2.1.99	B-72 2.1.99

NAME	DATE	DETAILS
→ B-40	17.6	P_0 5x T175 → 10 AMP FUNCTION↓ F_1
B-44	1.8	WAS USED ACCORDING TO MY RECORDS
B 45α+D	10.8	HEF P_4 T-175 → 2 AMP
B-46	15.8	HEF P_6 2x T775 → 4xAMP
→ B-72	2.1.99	KEEP FOR MARTIN - BAD PRODUCTION; BAD FUNCTION TO THROW AWAY 129 x 129

BOX 7 CANE 1

	1	2	3	4	5	6	7	8	9	
1-9	~~MEFM B-37~~	~~MEFM B-37~~	~~MEFM B-37~~	~~MEFM B-37~~	~~MEFM B-37~~	~~MEFM B-37~~	~~MEFM B-37~~	~~MEFM B-37~~	~~MEFMB-37~~	
10-18	~~MEFM B-37~~	~~MEFM B-37~~	~~MEFM B-37~~	~~MEFM B-37~~	~~MEFM B-37~~	~~MEFM B-37~~	~~MEF B-34~~	~~MEF B-34~~	~~MEF B-34~~	
19-27	GCTM1 13.7.98	GCTM1 13.7.98	GCTM1 3.7.98	GCTM1 3.7.98	GCTM2 3.7.98	GCTM1 3.7.98	GCTM1 3.7.98	GCTM2 3.7.98	GCTM13. 7.98	NOW 1-9
28-36	Cells? GCTM2 16.7.98	~~Cells?~~	~~Cells?~~	~~Cells?~~	~~Cells?~~	~~Cells?~~	~~Cells?~~	~~Cells?~~	~~Cells?~~	NOW 10-18
37-45	~~B-78 MEF~~	~~B-78 MEF~~	MEFM B-42	MEFM B-42	MEFM B-42	MEFM B-42	MEFM B-42	MEFM B-42	MEFMB-42	MEF-5 17.7.98 8 4 2 19-...
46-55	B-32 4.4	B-32 25.3	B-32 26.3	B-32 28.3	B-32 30.3	B-32 5.4	B-32 14.5	B-32 2.6.98	B-32 2.6.98	← POSNS 28-36
56-63	~~B-36 28.6~~	~~B-36 28.6~~		~~B-28 1.9~~	~~B-36 17.11~~				~~B-78 MEF~~	
64-72			B-75 P0 20.1.99	B-75 20.1	~~MEFM B-42~~	B-75 20.1	B-75 20.1	~~B-75 20.1~~	~~B-78 MEF~~	39-40
73-81	NS 6.12	NS 6.12	NS 6.12	NS 9.12	NS 9.12	NS II 15.12				BLANK 46—72 INC

NAME	DATE	DETAILS
→ B-37	10.6.98	MECHANICAL DERIVATION F1 3×10^6 CELLS/AMP × 15 P0
→ B-34		?
B-42	17.7	F1 P0 5× T-175 → 10×AMP THAW TO T-175
B-78		THAWED CELL FROM B-66 → WERE USED
→ B-36		?
B-75	20.1	2× 175 → 2×AMP/FLASK 1× 75 → AMP good function as fresh, 129X C57

B-71
P2 23.12.98
7→9

HES 2 → NOW 10-17

CAN 6 BOX 7 B-71 19.12

						P4	P4	P5	
1-9	B-71 24.12	B-71 24.12	B-77 26.4	B-60 2.11.98	B-60 2.11.98	B-60 2.11.98	B-60 2.11.98	B-60 2.11.98	B-60 2.11.98
10-18	B-77 26.4	B-77 26.4	HES-2 16.3.P4	HES-2 16.3.P4	B-61 11.11	B-61 11.11	B-61 11.11	B-61 11.11	HES-2 19.3 P5
19-27	HES-2 19.3 P5	HES-2 18.3 P5	HES-2 18.3 P5	HES-2 16.3 P4	HES-2 16.3 P4	B-64 26.11	B-64 26.11	B-64 26.11	B-64 26.11
28-36	~~B-65 7.12~~	~~B-65 7.12~~	~~B-65 7.12~~	~~B-65 7.12~~	B-64 7.12	B-64 7.12	B-64 7.12	B-64 7.12	B-64 7.12
37-45	B-64 7.12	B-64 7.12	B-77 26.4	B-76 29.3	B-76 29.3	~~B-77 26.4~~	~~B-66 8.12~~	~~B-66 8.12~~	B-76 29.3
46-54	B-76 29.3	B-76 29.3	B-73 18.1.99	B-73 18.1.99	B-73 18.1.99	~~B-70 16.12~~	B-70 16.12	B-70 16.12	B-70 16.12
55-63	B-70 16.12	B-70 16.12	B-69 17.12	B-69 17.12	B-69 17.12	B-68 18.12	B-68 18.12	B-68 18.12	B-68 18.12
64-72	B-71 19.12	B-71 19.12	B-71 19.12	B-71 19.12	B-73 6.1	B-73 6.1	B-73 6.1	B-73 6.1	B-76 29.3
73-81	B-68 23.12	B-71 23.12	B-71 23.12	B-71 23.12	B-71 24.12	endrew	endrew	~~B-36 6.98~~	cola

MEF

B-64 HEF P9
7.12 28-34

B-70 HEF
37-41

NAME	DATE	DETAILS
B-71	24.12	129 SVX129 P3 2×75 → 3 AMP GOOD AS FRESH
B-77	26.4	B-66 4×T-75 → 8 AMP P3 MOST PROBABLY WAS USED
B-60	2.11	F1 P3 4×175 + 1×75 → 9×AMP OVER CROWDNESS ×2 ALONG PASSAGES GOOD FUNCTION AS FRESH BEFORE
B-61	11.11	B-44 (WERE GOOD) → P3 2× 175 → 4× AMP → DID NOT FUNCTION VERY WELL
B-64	26.11 / 7.12	MEF 2× 175 → 4× AMP HEF THAWED P6 → REFROZEN P9 $3\frac{1}{2}$ T-175 → 7AMP
B-65		WAS ~~THA~~ DISCARDED
B-77		B-66 → WAS USED
B-66		WAS USED
B-73	6.1	129×C57 P0 2×175 → 4×AMP GOOD AS FRESH, QUITE POOR AS THAWED
B-70	16.12	HEF THAWED → REFREEZED P8 2×175 + 2×75 → 6 AMP
B-69	17.12	129 X129 P0 1×175 + 1×75 → 3 AMP GOOD AS FRESH AT P1 BUT BAD AFTER P2
B-68	18.12	129 X129 ONE EMBRYO FROZEN AT P2 2× 75 → 4 AMP

37-41

BOX 6 CANE 1 ,
Ben's Box

1-9	B-81 30.7	B-81 30.7	B-81 30.7	B-81 30.7	B-81 30.7	B-81 30.7	B-81 30.7	B-81 30.7	B-81 30.7
10-18	B-81 30.7	B-81 30.7	B-81 30.7	B-81 30.7	B-81 30.7	B-81 30.7	B-81 30.7	B-81 30.7	B-81 30.7
19-27	B-81 30.7	B-81 30.7	B-81 30.7	B-81 30.7	B-81 2.8	B-81 2.8	B-81 2.8	B-81 2.8	B-81 2.8
28-36	B-81 2.8	B-81 2.8	B-81 2.8	B-81 2.8	B-81 2.8	B-81 2.8	B-81 2.8	B-81 2.8	B-81 2.8
37-45	B-81 2.8	B-81 2.8	B-81 2.8	B-81 2.8	B-81 2.8	B-81 2.8	~~B-66~~	~~B-66~~	~~B-66~~
46-54	~~B-66~~	~~B-66~~	~~B-66~~	~~B-66~~		B-82 7.10	B-82 7.10	B-82 7.10	
55-63	~~B-36 28.6~~	~~B-36 28.6~~	~~B-36 28.8~~	~~B-36 2.9~~	~~B-36 17.11~~			HES-1 3.11.98	HES-1 3.11.98
64-72	HES-1 31.10.9	HES-1 1.11.98	HES-1 1.11.98	HES-1 1.11.98	HES-1 1.11.98	HES-1 2.11.98	HES-1 2.11.98	HES-1 2.11.98	HES-1 2.11.98
73-81	HES-1 29.10.9	HES-1 29.10.9	HES-1 29.10.9	HES-1 30.10.9	HES-1 30.10.9	HES-1 30.10.9	HES-1 30.10.9	HES-1 31.10.9	HES-1 31.10.9

NAME	DATE FROZEN.	
√B-81	30.7.99	129 X129 HIGH DENSITY A DAY FOLLOWING DERIVATION SPLIT 1:2 → FREEZING (P_0) FUNCTION - MODERATE (-) 9 vials.
~~B-36~~	~~?~~	
√B-82	7.10.99	129 x 129 Po 2 x 175 → 3 amp.
HES-1 P5	End oct Early nov 1998.	
~~B-81~~	~~2.8~~	~~129 x 129 AS ABOVE P_2~~ ~~5 x 175 → 20 AMP~~ DISCARDED.
√B-86	25.11.99	CBA x CBA Po. 4 x 175 → 8 amp.
√B-87	2.12.99	CBA x CBA Po 3 x 175 → 7 amp.
√B-88	2.12.99	129 x 129 Po.

BOX 1 (1/1) CANE 1

	1	2	3	4	5	6	7	8	9	10
A										
B		B-39 17.10.9	B-39 17.10.9	B-39 17.10.9	B-39 17.10.9	B-39 17.10.9	B-39 17.10.9			
C		B-39 20.10.9	B-39 20.10.9	B-39 20.10.9	B-39 20.10.9	B-39 20.10.9	B-39 20.10.9			
D		B-83 29.10	B-83 29.10	B-83 29.10	B-83 29.10	B-83 29.10	B-83 29.10	B-83 29.10	B-83 29.10	B-83 29.10
E		B-84 30.10	B-84 30.10	B-84 30.10	B-84 30.10	B-84 30.10			B-84 30.10	
F										
G							HES-2 13.4.99	HES-2 13.4.99	HES-2 13.4.99	~~B-66 1.2~~
H	HES-2 14.4	HES-2 14.4								
I			HES-2 15.5							
J										

✓B-39	✓17.10.99 ✓20.10.99	F~~[illegible]~~ P_2 3X 175 → 6 AMP P_3T-~~75~~ ×6 → 6 AMP IN BOTH CASES WERE NON CONFLUENT. THAW IN EVE[illegible] TO T[illegible] FUNCTION AS THAWED REASONABLE
✓B-83	29.10.99	129 × 129 P_0 4×175 + 1×75 → 9×AMP GOOD AS THAWED
✓B-84	30.10.99	129×129 P_0 2×175 2×75 } 6×AMP
✓B-86	26.11.99	As before P_0. 2×175 → 4 amp.
✓B-83	12.11.99	P 2 from P_0 above. 3×175 → 12 amp.

PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Benjamin E. Reubinoff, et al.	**Examiner:**	J. T. Woitach
Serial No.:	09/436,164	**Art Unit:**	1632
Filed:	November 9, 1999	**Docket:**	13164
For:	EMBRYONIC STEM CELLS		

Commissioner for Patents
Alexandria, VA 22313-1450

DECLARATION OF MARTIN F. PERA UNDER 37 C.F.R. §1.132

This is the exhibit marked as "Exhibit D" referred to in the Declaration of MARTIN F. PERA.

By: ____________________ Dated: 18/12/2003

RESULTS OF TESTS IN MAY 99 TO GO AFTER HERE

~~RO~~BLAST FEEDERS MAY 99 ⇒ 14.1.02.

B-77. – Used many times – must be okay. R6 B7 Bk

B-76. – R6 B7 POS 41. – Used many times.

B-66 R6 B7 Posn E43. Later frozen as B-78. P_3
⇒ used many times (11.6.99)

B-68 – ? used only once

B-75 – ? Used only once.

B-80 – New prep on 19/7/99. ⇐ No good ⇐ discarded
1 foetus only.

B-78 R1 B7 Posn 37. ⇐ Used many times.

B-79 New prep on 28/6/99. ? Not good?

B-81 – New prep on 28/7/99.

B-82 New prep on 5/10/99. ⇐ Discarded?
poor growth.

B-39 – okay but not brilliant.

B-83 – Good still using.

B-84
B-85
B-86
B-87
B 88
⇐ All new preparations
– not tested as feeders as yet.

PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Benjamin E. Reubinoff, et al. **Examiner:** J. T. Woitach

Serial No.: 09/436,164 **Art Unit:** 1632

Filed: November 9, 1999 **Docket:** 13164

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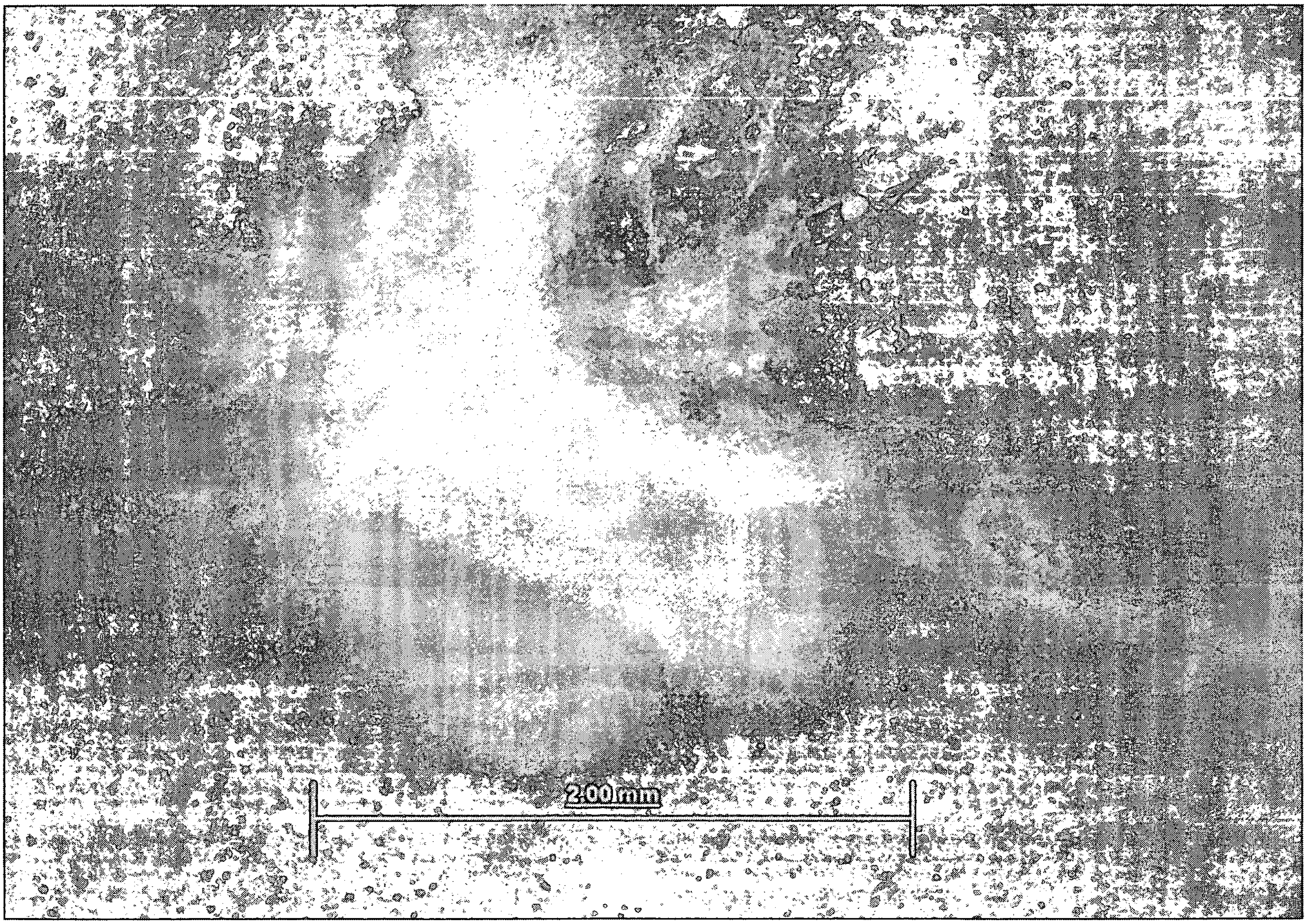
DECLARATION OF MARTIN F. PERA UNDER 37 C.F.R. §1.132

This is the exhibit marked as "Exhibit E" referred to in the Declaration of MARTIN F. PERA.

By: ____________________ Dated: 18/12/03


2.00 mm